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ASHG/ACMG STATEMENT
Measurement and Use of Total Plasma Homocysteine

American Society of Human Genetics/American College of Medical Genetics Test and Technology Transfer Committee Working Group

Summary

Hyperhomocysteinemia, which is a recognized independent risk factor for premature vascular occlusion, is defined as a fasting total plasma homocysteine (tHcy) level >15 μM. There may also be graded increased risks for persons with tHcy concentrations of 10–15 μM. The measurement of tHcy requires precise sample collection, immediate separation and freezing of plasma, and referral to a specialized laboratory. The etiologies of hyperhomocysteinemia are complex and involve both genetic and environmental factors. Because the inappropriate supplementation of involved cofactors can be harmful, it is important to identify the cause of hyperhomocysteinemia prior to treatment.

Introduction

It has been known for some time that the concentrations of homocystine and homocysteine are increased in blood and urine in (*a*) cystathionine β-synthase deficiency, (*b*) methylenetetrahydrofolate reductase (MTHFR) deficiency, and (*c*) defects in the synthesis of cobalamin (vitamin B_{12}) cofactors. The large increases in the concentrations of homocystine in these conditions are easily detected by the ion-exchange chromatography methods used to measure amino acids in most laboratories. In recent years, however, it has been found that much smaller increases in total plasma homocysteine (tHcy) concentrations are found in pyridoxine, cobalamin, and folate deficiencies and, under certain conditions, in subjects with a heat-labile form of MTHFR. Furthermore, even a slightly increased tHcy level is a risk factor for the development of coronary, cerebral, and peripheral vascular disease, as well as venous thrombi, and the increased risk is independent of other risk factors such as hyperlipidemia, hypertension, diabetes mellitus, and smoking. Laboratories are thus now being asked to distinguish between normal tHcy concentrations and those that put patients at increased risk for these conditions. The following paragraphs review the methods available to make this distinction, and they provide some guidelines to define and manage hyperhomocysteinemia.

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Measurement of tHcy

The structures of homocysteine, homocystine (homocysteine-homocysteine disulfide), and homocysteine-cysteine disulfide are shown in figure 1. Approximately 70% of the homocysteine in plasma or serum is bound to cysteine residues in albumin and other plasma proteins, and only trace amounts exist as free homocysteine and in homocystine and homocysteine-cysteine disulfide. The methods that are used to measure free amino acids in most laboratories cannot detect the small amounts of these compounds present in normal blood, nor can they detect the small increases that may put patients at increased risk for premature cardiovascular disease. That level of sensitivity can only be achieved by addition of a reducing agent, such as dithiothreitol (DTT) or beta-mercaptoethanol, to freshly obtained plasma, to release homocysteine from plasma proteins and other thiols, before direct measurement of what is then termed "tHcy."

The accuracy and reproducibility of tHcy testing is dependent, first, on precise sample collection and handling; the blood samples must be placed on ice immediately to prevent the release of free homocysteine from erythrocytes, and samples should be centrifuged, and the plasma frozen, ⩽30 min after collection. The tHcy is then stable for relatively long periods of time (i.e., months).

All of the methods that have been developed to measure tHcy after addition of a reducing agent give similar normal values of 5–15 μmol/liter (or μM) (Ueland et al. 1993). Although ion-exchange chromatography on an amino acid analyzer can be used to do this, most laboratories that process large numbers of samples use methods based on high-performance liquid chromatog-

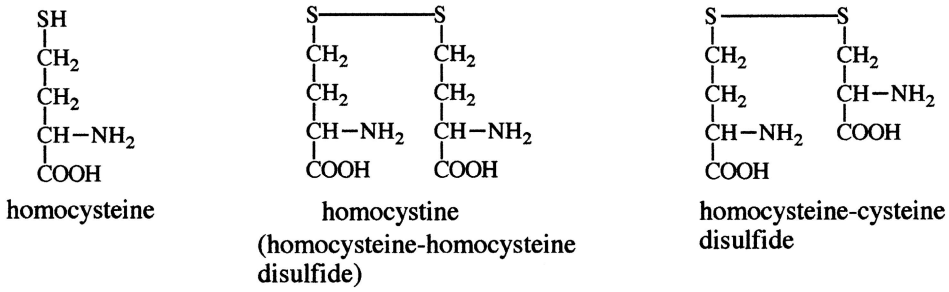

Figure 1 Molecular structures of homocysteine, homocystine, and homocysteine-cysteine disulfide

raphy (HPLC) or stable isotope–dilution gas chromatography–mass spectrometry (GC-MS).

Although methods to measure tHcy by HPLC are still evolving, most of them are designed to detect fluorescent derivatives of homocysteine bound covalently to a chromophore. The most easily automated of these uses sample reduction with tri-*n*-butylphosphine, followed by chromatography of sulfhydril-containing compounds such as the *s*ulfo*b*enzooxa*d*iazole (SBD) derivatives (Araki and Sako 1987). Other fluorescent derivatives may also be used, but the *o*-phthaldehyde derivative of homocysteine is less stable and must be injected into the HPLC shortly after it is made, and monobromobiamine, another derivatizing reagent (Jacobsen et al. 1989, 1994), is itself fluorescent and can produce chromatographic peaks that may complicate the analysis. Other HPLC-based methods, such as those that use a gold-mercury electrode to detect underivatized thiols or that convert homocysteine to S-adenosyl-methionine, are used less frequently.

tHcy concentration may also be measured by stable isotope–dilution GC-MS with selected ion monitoring. In this system, deuterated (usually) homocysteine is added to the specimen as an internal standard before reduction and derivitization, and homocysteine is measured after capillary GC-MS, by measurement of the extent to which the internal standard is diluted by homocysteine in the sample. Although the high cost of the instrumentation has made the use of this system less common than the use of HPLC, a relative advantage is that methionine, methylmalonic acid, and cystathionine, compounds that are important in differential diagnosis, can be measured at the same time (Stabler et al. 1987, 1993).

Clinical Correlates

The Hordaland study (Nygaard et al. 1995) and the physicians' health trial (Stampfer et al. 1992), as well as a metanalysis of 27 studies (Boushey et al. 1995), found that tHcy values >15 μM confer an increased risk for premature vascular occlusion and that values of 10–15 μM may confer a graded risk. It is appropriate to use 15 μM as an initial threshold for the diagnosis of hyperhomocysteinemia, although the value may be revised downward as more data are collected. Because of both the inherent complexity of testing and the importance of correct sample collection and processing, a patient with a single elevated tHcy value should be retested after a fast of $\geqslant$8 h.

The basis of fasting tHcy levels >15 μM should always be determined. It is important to measure serum and erythrocyte folate, serum cobalamin, and serum and/or urine methylmalonic acid levels. Supplementation with folate alone can obscure a diagnosis of cobalamin deficiency, by masking hematologic findings while allowing neurological damage to progress (Savage and Lindenbaum 1995). Genetic factors that may contribute to elevations of tHcy include heterozygosity for severe forms of several inborn errors—among them cystathionine β-synthase deficiency, MTHFR deficiency, and methionine synthase deficiency—and the cblC, cblD, cblE, and cblF defects of methylcobalamin biosynthesis. In addition, common polymorphisms for MTHFR may contribute to elevated tHcy, particularly when nutritional folate status is marginal.

Therapy should be based on knowledge of etiology and pathogenesis. Relatively small (e.g., $\leqslant$1 mg/mo) doses of parenteral cobalamin can be given to persons with cobalamin malabsorption or deficiency, whereas patients with blocks in methylcobalamin biosynthesis may require milligram doses daily. Similarly, folate deficiency secondary to sprue can be treated with oral folate, whereas patients with hereditary folate malabsorption require reduced folates in high doses given parenterally. Deficiency states will probably require greater amounts than the RDA, for both vitamins. The efficacy of therapy can be monitored by measurement of tHcy and, if cobalamin deficiency or (appropriate) biosynthetic defect is present, of urine or blood methylmalonic acid.

Disclaimer

This guideline is designed primarily as an educational resource for medical geneticists and other health-care providers, to help them provide quality medical genetic services. Adherence to this guideline does not necessarily ensure a successful medical outcome. This guideline should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed toward obtaining the same results. In determining the propriety of any specific procedure or test, the geneticist should apply his or her own professional judgment to the specific clinical circumstances presented by the individual patient or specimen. It may be prudent, however, to document in the patient's record the rationale for any significant deviation from this guideline.

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